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(74) Agents: HUGHES, Ivor, M. et al.; Suite 200, 175 Commerce Valley Drive West, Thornhill, Ontario L3T 7P6 (CA).			
(54) Title: TREATMENT OF DISEASE AND CONDITIONS ASSOCIATED WITH MACROPHAGE INFILTRATION IN PARTICULAR STROKE AND MYOCARDIAL INFARCTION			
(57) Abstract <p>Use of hyaluronic acid and/or salts thereof for the treatment of a human having a disease or condition characterized by macrophage, neutrophile or other white blood cell infiltration into an area damaged by the disease or condition, wherein said use comprises the use of an effective amount of hyaluronic acid and/or salts thereof for a period of time until such use is no longer required.</p>			

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INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	see page 61, paragraph 1; claims 1-6,46-80,82,139; examples XX,XL see page 59, paragraph 4-6 see page 34, line 5 - page 35, line 30 ---	1-10,13, 15-33
X	EP,A,0 557 118 (NORPHARMCO INC) 25 August 1993 see page 2-3; claims 1-18 ---	1,2,11, 12,15, 16,25,26
Y	US,A,4 725 585 (WENGE PER S W ET AL) 16 February 1988 see the whole document ---	1-10, 15-33
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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- *'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *'&' document member of the same patent family

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INTERNATIONAL SEARCH REPORT

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Information on patent family members

International Application No

PCT/CA 95/00467

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<p>(54) Title: TREATMENT OF DISEASE AND CONDITIONS ASSOCIATED WITH MACROPHAGE INFILTRATION IN PARTICULAR STROKE AND MYOCARDIAL INFARCTION</p> <p>(57) Abstract</p> <p>Use of hyaluronic acid and/or salts thereof for the treatment of a human having a disease or condition characterized by macrophage, neutrophile or other white blood cell infiltration into an area damaged by the disease or condition, wherein said use comprises the use of an effective amount of hyaluronic acid and/or salts thereof for a period of time until such use is no longer required.</p>			

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TITLE OF INVENTION

TREATMENT OF DISEASE AND CONDITIONS ASSOCIATED WITH MACROPHAGE INFILTRATION IN PARTICULAR STROKE AND MYOCARDIAL INFARCTION

FIELD OF INVENTION

5 This invention relates to the treatment of diseases and conditions characterized by leukocyte [e.g. white blood cells] infiltration into the area damaged by the disease or condition for example in oxygen and/or glucose deprived tissue in the human body. This invention finds one application in the treatment of
10 stroke. This invention finds another application in the treatment of infarcts (myocardial infarction). This invention also finds application in the treatment of any disease or condition characterized by leukocyte [e.g. white blood cells] infiltration to the area of damaged tissue of the body.

15 BACKGROUND OF THE INVENTION

When tissue (and the individual cells) are deprived of oxygen and/or glucose, the cells and consequently the tissue made up by the cells are damaged. As a result, among other responses, an inflammatory response (reaction) is set up in the area (site) of the
20 damage. This inflammatory response includes, among other responses, the migration of inflammatory cells (for example macrophages, neutrophils, and other white blood cells) to the site of the damage.

For example, during a stroke or infarct (e.g. myocardial
25 infarction, heart attack), whatever the cause, the blood supply in the blood vessels is impaired and diminished. The consequences include deprivation of oxygen and glucose resulting in, at the very least, damage to the deprived areas.

This damage sets, among other responses, an
30 inflammatory response in the area damaged with the consequent migration of inflammatory cells (macrophages, neutrophils and other white blood cells). Because of the damage (trauma) to the site, prostaglandin synthesis also increases.

Cerebral deprivation of oxygen and glucose in premature
35 birth follows the same scenario - a deprivation of oxygen to the brain of the infant which sets up an inflammatory response (migration of inflammatory cells (for example macrophages, neutrophiles and other

white blood cells).

It is therefore an object of this invention to provide pharmaceutical compositions (for example, injectibles (sterile)), methods of treatment, and new uses for known chemicals which 5 reduce the damage caused to the tissue and cells (when the compositions are employed), for example, resulting from a stroke, infarct (myocardial infarction) and/or any disease or condition characterized by an inflammatory response for example by macrophage, neutrophil or other white blood cell infiltration or 10 migration into the area of the damage.

It is a further object of this invention to provide pharmaceutical compositions, methods of treatment and new uses of known chemicals which downregulate the cells' activity (macrophages, neutrophils and other white cells) and thus modify 15 (alter) the body's anti-inflammatory response.

Further and other objects of the invention will be realized by those skilled in the art from the following summary of invention and detailed description of embodiments thereof.

SUMMARY OF THE INVENTION

20 According to one aspect of the invention, when tissue and thus cells are damaged for example by being deprived of glucose and/or oxygen, the activity of inflammatory cells (for example macrophages, neutrophils and other white blood cells) will be modulated (for example their migration into the area (to the site) of 25 damage will be diminished), by the administration of an effective amount of hyaluronic acid and/or salts thereof (for example sodium hyaluronate) at the time the area is being damaged (as for example at the time of a stroke or infarct or other disease or condition which is characterized by macrophage, neutrophile or other white blood cell 30 infiltration into the area of the tissue damaged by the condition and/or disease or a short time thereafter. While Applicant should not be limited to the following mechanism of action of the invention, Applicant believes that the administration of the hyaluronic acid and/or salts thereof (e.g. sodium salt) blocks, for example by binding 35 with, the hyaluronic acid (HA) receptors of the inflammatory cells (for example macrophages, neutrophiles and other white blood cells), thus blocking their migration into the area of the damaged tissue.

The preferred form of hyaluronic acid is sodium hyaluronate having a molecular weight less than 750,000 daltons for example 10,000 - 300,000 daltons.

To supplement this inhibition (blockage) of the inflammatory response, (which inflammatory response causes the migration or infiltration of the macrophages, neutrophiles or other white blood cells into the damaged area), NSAIDS may also be given with the form of hyaluronic acid which blocks (for example binds with) the HA receptors. Thus inhibition of prostaglandin synthesis can be achieved.

The hyaluronic acid and/or salts (preferably sodium hyaluronate having a molecular weight less than 750,000 daltons) may be utilized at varying doses (depending upon the method of administration) from 1-10 mg/kg body weight to 15 - 20 mg/kg of body weight or more, for example a dose in excess of 25 mg/kg and more to over 3000 mg/70 kg person. In adult human (and adult rats) excess amounts of the form of hyaluronic acid are tolerated, however in rat neonates, excess can and does cause damage.

Thus and according to another aspect of the invention when an NSAID for example indomethacin (dissolved in n-methyl glucamine (nmg)) or other NSAID is administered with greater than 200 mg hyaluronic acid per 70 kg person with 1 - 2 mg/kg body weight of the NSAID (in one instance indomethacin and NMG), no major toxic side effects occur such as gastro-intestinal distress, neurological abnormalities, depression, etc., even at elevated amounts of indomethacin (if necessary). If the amount of hyaluronic acid is decreased below that amount, the usual side effects of using an NSAID may begin to reoccur. The same can be said with other therapeutic agents, no major toxic side effects occur with the administration of greater than 200 mg hyaluronic acid (e.g., sodium hyaluronate per 70 kg-person).

Preferably (and on the basis of tests performed) each preferred dosage amount of the preferred form of hyaluronic acid, sodium hyaluronate, should be in the order of about 10-25 mg/kg body weight, for example in the order of about 1800 mg/70 kg person if administered subcutaneously for example in the back. Intravenous dosing could employ smaller (lesser) dose amounts of the form of

hyaluronic acid. Preferred amounts are 10-20 mg./kg of body weight of a human.

Presently testing in neonate rats reveals that suitable dosage amounts will establish a concentration volume of the chosen form of hyaluronic acid (for example sodium hyaluronate) of about 3 mg/ml of blood in an adult human. When administered, an amount of between about 10mg of, for example, sodium hyaluronate/kg and about 25mg of, for example, sodium hyaluronate/kg of body weight of an adult human. More recent tests with rats neonates, achieved levels of hyaluronic acid (12 hours after administration subcutaneously in the neonates) at 10 mg/kg in their blood. These amounts can be adjusted up or down as would be deemed preferable in the circumstances.

The administration preferably starts at the time of the disease or condition (for example stroke or myocardial infarction) occurring or shortly thereafter (within 24 hours) and continues until such time as not required. Preferably the level achieved is maintained in the blood. For example a level of 15 mg/kg of body weight is established in the blood, by initial intravenous administration. Then that level is maintained by for example subcutaneous administration (for example, by subcutaneous injection).

NSAIDS may be given at the same time in effective amounts (e.g. 1-2 mg/kg of body weight in the case of indomethacin).

Drugs for the treating of a myocardial infarction or stroke may also be administered such as a clot dissolving drug. Clot dissolving drugs comprise TPA, Streptokinase (proteolytic product), Urokinase and the like. Other available drugs are the NSAID acetylsalicylic acid (aspirin), beta blockers, heparin, a plasminogen activator, and other suitable drugs.

In addition to the NSAIDS, other drugs (where a stroke is or has occurred) may be administered with the form of hyaluronic acid (for example sodium hyaluronate having a molecular weight less than 750,000 daltons for example 300,000 daltons) with or without the NSAIDS. These drugs would be in their expected amounts. These drugs may be for example the same as above and may also comprise anti-platelet drugs for example those which alter the platelet function

(prevent aggregation) and prevent thrombosis formation (clots).

One form of hyaluronic acid and/or salts thereof (for example sodium salt) and homologues, analogues, derivatives, complexes, esters, fragments, and sub units of hyaluronic acid,

5 preferably hyaluronic acid and salts and thereof suitable for use with Applicant's invention is a fraction supplied by Sterivet Laboratories Limited. One such fraction is a 15 ml vial of Sodium hyaluronate 20 mg/ml (300 mg/vial - Lot 2F3). The sodium hyaluronate fraction is a 2% solution with a mean average molecular weight of about 225,000.

10 The fraction also contains water q.s. which is triple distilled and sterile in accordance with the U.S.P. for injection formulations. The vials of hyaluronic acid and/or salts thereof may be carried in a Type 1 borosilicate glass vial closed by a butyl stopper which does not react with the contents of the vial.

15 The fraction of hyaluronic acid and/or salts thereof (for example sodium salt) may comprise the following characteristics:

a purified, substantially pyrogen-free fraction of hyaluronic acid obtained from a natural source having at least one characteristic selected from the group consisting of the following:

20 (i) a molecular weight within the range of 150,000-225,000;

(ii) less than about 1.25% sulphated mucopolysaccharides on a total weight basis;

(iii) less than about 0.6% protein on a total weight basis;

25 (iv) less than about 150 ppm iron on a total weight basis;

(v) less than about 15 ppm lead on a total weight basis;

30 (vi) less than 0.0025% glucosamine;

(vii) less than 0.025% glucuronic acid;

(viii) less than 0.025% N-acetylglucosamine;

(ix) less than 0.0025% amino acids;

(x) a UV extinction coefficient at 257 nm of less than 35 about 0.275;

(xi) a UV extinction coefficient at 280 nm of less than about 0.275;

(xii) a pH within the range of 7.3-7.9.

Preferably the hyaluronic acid is mixed with water and the fraction of hyaluronic acid fraction has a mean average molecular weight within the range of 150,000-225,000. More 5 preferably the fraction of hyaluronic acid comprises at least one characteristic selected from the group consisting of the following characteristics:

- (i) less than about 1% sulphated mucopolysaccharides on a total weight basis;
- 10 (ii) less than about 0.4% protein on a total weight basis;
- (iii) less than about 100 ppm iron on a total weight basis;
- 15 (iv) less than about 10 ppm lead on a total weight basis;
- (v) less than 0.00166% glucosamine;
- (vi) less than 0.0166% glucuronic acid;
- (vii) less than 0.0166% N-acetylglucosamine;
- (viii) less than 0.00166% amino acids;
- 20 (ix) a UV extinction coefficient at 257 nm of less than about 0.23;
- (x) a UV extinction coefficient at 280 nm of less than 0.19; and
- (xi) a pH within the range of 7.5-7.7.

25 Other forms of hyaluronic acid and/or its salts, and homologues, derivatives, complexes, esters, fragments and sub units of hyaluronic acid may be chosen from other suppliers, for example those described in the prior art documents previously referred to. In addition Applicants have propose sodium hyaluronate produced and 30 supplied by LifeCore™ Biomedical, Inc. having the following specifications

<u>Characteristics</u>	<u>Specification</u>
Appearance	White to cream
	coloured particles
35 Odor	No perceptible odor

- 7 -

Viscosity Average < 750,000 Daltons
Molecular Weight

5 UV/Vis Scan, 190-820 nm Matches reference scan
OD, 260 nm < 0.25 OD units

10 Hyaluronidase Sensitivity Positive response
IR Scan Matches reference

pH, 10mg/g solution 6.2 - 7.8

15 Water 8% maximum
Protein < 0.3 mcg/mg NaHy
Acetate < 10.0 mcg/mg NaHy

20 Heavy Metals, maximum ppm
As Cd Cr Co Cu Fe Pb Hg Ni
2.0 5.0 5.0 10.0 10.0 25.0 10.0 10.0 5.0

25 Microbial Bioburden Non observed
Endotoxin < 0.07EU/mg NaHy

30 Biological Safety Testing Passes Rabbit Ocular
Toxicity Test
The administration may take place subcutaneously,
intravenously or by injection.

DETAILED DESCRIPTION OF EMBODIMENTS

35 The invention will now be illustrated with respect to the
following example.

Experiment 1

Seven day old Fischer rat neonates were injected

subcutaneously with sodium hyaluronate (MW 300,000 daltons) in the back 1/2 hour before the operation with 0.6 mg of sodium hyaluronate /60ul/animal. Animals were then injected once a day for seven days after the operation and euthanized 14 days after the 5 operation. The right carotid artery was ligated for 1 hour (induced stroke). The animals were then placed in incubators containing 8% oxygen. (The left side was not tied off and provided a suitable control.) Brain damage was readily produced in control animals by 10 day 4 and 7 as determined by nissl staining (for nerves) and for gliosis including increased staining for GFAP, connexin 43, and macrophages (ED-1 epitope). As well increased staining for hyaluronan receptors was observed with CD44 increased in macrophages and astrocytes while RHAMM was increased in neuronal 15 cells and subsets of macrophages. As well damage was observed morphologically where neuronal loss was evident and the right half of the brain had collapsed. Animals treated with HA for 7 days were euthanized at 14 days (as were controls run with these experiments) and none of the above parameters were positive. That is there is no evidence of brain collapse, neuronal loss, macrophage influx or 20 increase in the expression of gliotic proteins or hyaluronan receptors. The hyaluronan treated animal's brains appeared morphologically normal, although functional test for neuronal activity have not yet been done. In the absence of any obvious morphological changes, extensive functional damage would not be expected.

25 The dose used for each animal was 0.6mg of sodium hyaluronate (Molecular Weight 300,000 daltons) per 23g animal by subcutaneous administration so for a human of 70 kg this would mean a 1.8g subcutaneous dose/person. An intravenous dose would be smaller.

30 There are approximately 85 cc blood/kg in humans. Therefore an average adult would have about 6000 cc (70 kg person). Thus the concentration achieved is in the order of 0.3 mg of sodium hyaluronate/1 ml or cc of blood. Administration to achieve this concentration is in the order of 25 mg/kg of body weight for humans.

35 Experiment 2

Experiment 2 repeated Experiment 1 only the right carotid artery was ligated for 3 hours (2 hours longer than the 1 hour

specified in Experiment 1) to accrue more brain necrosis. In Experiment 2 the same amount of sodium hyaluronate (HA) (0.6 mg of sodium hyaluronate) was administered to each neonate (regardless of the actual weight of the neonates) and each animal received an 5 injection of sodium hyaluronate subcutaneously at the time of the operation.

Twelve hours after subcutaneous administration, blood levels of 15-20 mg/kg of HA are obtained. Continued analysis of blood levels indicates that HA levels remain at 15 mg/kg for 24 hours.

10 After the operation, we continued to inject HA in the same dosage amounts every 24 hours for 7 days. At no time during the 7 days did the HA levels drop below 15 mg/kg.

The brains of the animals (including controls) were examined at 2 weeks.

15 Of the three animals injected with HA, the brains were in the same condition as the brains of the neonates administered with HA in Experiment 1. The one control suffered excessive brain damage. As a result of these tests, we have concluded that dose amounts of as low as 1 mg/kg of body weight of the animal will be therapeutic (e.g. 20 for blocking the infiltration of macrophages, neutrophiles and other white blood cells into the area (to the site) of the stroke. Dose amounts of 10 mg or more of HA/kg of the animal (human) are preferred for example 10-20 mg/kg of body weight.

Experiment 3

25 While humans and adult rats are able to tolerate excesses of sodium hyaluronate (HA), rat neonates are not as tolerant. Thus, in Experiment 3, where the rate neonates were smaller, dosage amounts of 25 mg/kg of HA, administered in Experiment 3, resulted in damage to the brains of the neonates.

Experiment 4

Four rats were each exposed to isoproterenol to induce myocardial infarction (heart attack) in each. The administration of isoproterenol for inducing an infarct is a commonly known technique as would be understood by persons skilled in the act and is not elaborated herein. Each of two of the rats was immediately after the infarct injected with sodium hyaluronate (Molecular Weight 300,000 daltons) (HA) in the amount of 15 mg/kg.

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The subcutaneous injections continued for seven days (one subcutaneous injection each day). Twelve hours after the initial injection, the blood levels of HA were 10 mg/kgm body weight in the blood system of each rat.

5 The other two rats were immediately after the induction of the infarct each injected subcutaneously with saline (as a control). The subcutaneous injections continued for seven days, one injection per day. The rats were then sacrificed. In the saline-treated animals, heart tissue was necrotic with massive amounts of 10 accumulated white cells. In the HA-treated rats, no damage was observed in the heart tissue and no white cells were apparent (as determined by E0-1 staining of frozen sections).

15 As a result, we have concluded that dosage amounts of 10 - 25 mg/kg of body weight administered to humans is appropriate. While lesser amounts can still be therapeutic, they will not give optimal results. Optimal results are the goal in the treatment of each of a stroke and myocardial infarction.

20 Preferably the chosen dosage amount of the HA is initially given intravenously to establish the desired levels of HA in the blood. Thereafter, these levels are maintained for example by administration subcutaneously (subcutaneous injection). Preferred blood levels appear to be starting in the order of 10 mg/kg.

25 As many changes can be made to the preferred embodiments of the invention without departing from the scope of the invention, it is intended that all material contained herein be interpreted as illustrative of the invention and not in a limiting sense.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE AS FOLLOWS:

1. Use of hyaluronic acid and/or pharmaceutically acceptable salts thereof for the treatment of a human having a disease or condition characterized by macrophage, neutrophile or other white blood cell infiltration into an area damaged by the disease or condition, wherein said use comprises the use of an effective amount of hyaluronic acid and/or salts thereof for a period of time until such use is no longer required.
2. The use of claim 1 wherein the effective amount of the form of hyaluronic acid is in the order of about 10 mg to about 25 mg per kilogram of body weight.
3. The use of claim 1 or 2 wherein the disease or condition is a stroke.
4. The use of claim 1 or 2 wherein the disease or condition is a myocardial infarction.
5. The use of claim 1 or 2 wherein the use starts within about 24 hours of the detection of the disease or condition.
6. The use of claim 1, 2, 3 or 4 wherein the hyaluronic acid or pharmaceutically acceptable salts thereof is sodium hyaluronate having a molecular weight less than 750,000 daltons.
7. The use of claim 1, 2, 3 or 4 wherein the hyaluronic acid and/or pharmaceutically acceptable salts thereof is sodium hyaluronate having a molecular weight of about 300,000 daltons.
8. The use of claim 1 wherein the concentration of the form of hyaluronic acid in a human after administration is at least of 3 mg/ml of blood.
9. The use of claim 1, 2, 3, 4, 5 or 6 further comprising

administration of an effective amount of a medicine selected from an NSAID, an anti-stroke drug, clot dissolving drug, Beta Blocker, acetylsalicylic acid, streptokinase, anti-platelet drugs, heparin or a plasminogen activator or combinations thereof.

5

10. The use of claim 1, 2, 3 or 4 wherein the blood levels of the form of hyaluronic acid is greater than 10 mg/kg of body weight.

10

11. An injectable sterile pharmaceutical formulation comprising in injectable dosage form at least 10 mg/kg of the human to whom the injectable is to be administered of hyaluronic acid and/or a pharmaceutically acceptable salt thereof such as sodium hyaluronate, having a molecular weight less than 750,000 daltons in a suitable vehicle.

15

12. The injectable formulation of claim 11 wherein the Molecular Weight of the form of hyaluronic acid is in the order of 300,000 daltons.

20

13. The injectable of claim 11 or 12 further comprising an anti-stroke drug, a clot dissolving drug, an NSAID, a Beta Blocker, acetylsalicylic acid, streptokinase, anti-platelet drugs, heparin, or a plasminogen activator or combinations thereof.

25

14. The injectable of claim 11 or 12 wherein the amount of the form of hyaluronic acid is about 25 mg/kg of the human for whom the injectable is to be administered.

30

15. A method of treating a human having a disease or condition characterized by macrophage, neutrophile or other white blood cell infiltration into the area damaged by the disease or condition, the method comprising administering to the human an effective amount of hyaluronic acid and/or pharmaceutically acceptable salts thereof for a period of time until the administration is no longer required.

35

16. The method of claim 15 wherein the effective amount of

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the form of hyaluronic acid is in the order of about 10 mg to about 25 mg per kilogram of body weight.

17. The method of claim 16 wherein the disease or condition
5 is a stroke.

18. The method of claim 16 wherein the disease or condition
is myocardial infarction.

10 19. The method of any of claims 15 or 16 wherein the
treatment starts within about 24 hours of the detection of the disease
or condition and continues until no longer required.

15 20. The method of any of claims 15, 16, 17 or 18 wherein the
hyaluronic acid and/or pharmaceutically acceptable salts thereof is
sodium hyaluronate having a molecular weight less than 750,000
daltons.

20 21. The method of claim 15, 16, 17 or 18 wherein the
hyaluronic acid and/or pharmaceutically acceptable salts thereof is
sodium hyaluronate having a molecular weight in the order of
300,000 daltons.

25 22. The method of claim 15 wherein the concentration of the
form of hyaluronic acid in a human after administration is at least 3
mg/ml of blood.

30 23. The method of claim 15, 16, 17, 18, 19 or 20 further
comprising administration of an effective amount of a medicine
selected from an NSAID, an anti-stroke drug as a clot dissolving drug,
Beta blocker acetylsalicylic acid, streptokinase anti-platelet drugs,
heparin or a plasminogen activator or combinations thereof.

35 24. The method of claim 15, 16, 17 or 18 wherein the blood
levels of the form of hyaluronic acid achieved is greater than about
10 mg/kg of body weight of the person.

25. Use of hyaluronic acid and/or pharmaceutically acceptable salts thereof for the manufacture of a dosage amount of a pharmaceutical composition for the treatment of a human having a disease or condition characterized by macrophage, neutrophile or other white blood cell infiltration into an area damaged by the disease or condition, wherein said use comprises the use of an effective amount of hyaluronic acid and/or pharmaceutically acceptable salts thereof.

10 26. The use of claim 25 wherein the effective amount of the form of hyaluronic acid is in the order of about 10 mg to about 25 mg per kilogram of body weight.

15 27. The use of claim 25 or 26 wherein the disease or condition is a stroke.

28. The use of claim 25 or 26 wherein the disease or condition is a myocardial infarction.

20 29. The use of claim 25, 26, 27 or 28 wherein the hyaluronic acid and/or pharmaceutically acceptable salts thereof is sodium hyaluronate having a molecular weight less than 750,000 daltons.

25 30. The use of claim 25, 26, 27 or 28 wherein the hyaluronic acid and/or pharmaceutically acceptable salts thereof is sodium hyaluronate having a molecular weight of about 300,000 daltons.

30 31. The use of claim 25 wherein the concentration of the form of hyaluronic acid in a 70kg human after administration will be at least 3 mg/ml of blood.

35 32. The use of claim 25, 26, 27, 28 or 29 further comprising administration of an effective amount of a medicine selected from an NSAID, an anti-stroke drug, clot dissolving drug, Beta Blocker, acetylsalicylic acid, streptokinase, anti-platelet drugs, heparin or a plasminogen activator or combinations thereof.

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33. The use of claim 25, 26, 27 or 28 wherein the blood levels of the form of hyaluronic acid will be greater than 10 mg/kg of body weight after administration of the dosage amount.

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